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A Comparative Evaluation of Smear Layer Removal by Using EDTA, Apple Cider Vinegar, and Normal Saline as

Root Canal Irrigants: An In Vitro Scanning Electron Microscopic Study

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Abstract

Aim: The purpose of the following study is to evaluate and compare the efficacy of 17% EDTA, apple cider vinegar and 0.9% normal saline using scanning electron microscopy in removing smear layer from root canal.

Materials and Methods: Root canals of 15 straight single-rooted and decoronated single canal maxillary central incisors were instrumented using crown-down technique and then equally divided into three groups on basis of irrigation solutions used: irrigation with 17% EDTA, apple cider vinegar and 0.9% normal saline into Group I, II, III respectively. Samples were then longitudinally sectioned and examined under scanning electron microscope using scores from 1 to 3. Data was analyzed using Statistical Package for Social Sciences (SPSS), version 15.0. Kruskal–Wallis analysis of variance (ANOVA) followed by Mann-Whitney U test were used for intragroup and intergroup comparisons respectively. The level for significance was set at 0.05.

Results: Minimum means score was observed in Group I and Group II at coronal and apical locations. Difference in scores between the groups was found to be statistically significant for all three locations as well as for overall assessment (P < 0.05).

Conclusion: Apple cider vinegar has comparable smear layer removal efficacy with 17% EDTA solution and normal saline from the coronal, middle and apical third of the root canals. Hence, the biocompatibility of Apple cider vinegar over 17% EDTA is an added advantage.

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Introduction

The conquest of endodontic treatment depends directly on cleaning, shaping, and proper obturation with adequate seal of the root canal. However, during the process of cleaning and shaping, the instrument that comes in contact with the root canal walls causes formation of the smear layer. Complete debridement includes smear layer removal which helps to achieve a successful outcome of the root canal treatment.[1] Based on recent studies, bacteria has the ability to endure and multiply in the smear layer. They can even penetrate deep into dentinal tubules, thus preventing the penetration of irrigants and intracanal medicaments into the dentinal tubules. In addition, smear layer might interfere with the antimicrobial properties of medicaments and adaptation ability of sealer cement onto the walls. Therefore it is essential to remove smear layer from root canal to achieve accomplishment of the treatment. [2][3].

Moreover, Ultrasonic instruments, lasers, and chelating agents have been used for the purpose of removing the organic and inorganic components of the smear layer [4]. Several studies have shown that the use of a sodium hypochlorite (2.5-5%) in association with EDTA (10-17%) is particularly effective with tissue dissolution properties. Therefore the combination is considered to be a gold standard in terms of smear layer removal [2]. However, EDTA can cause erosion of dentin, depending on its application time and concentration. 1%, 5%, 10%, and 15% of EDTA when used for 1 min caused erosion of the root dentin, as reported by **Sen et al. (2009)[5]**. Thus the quest for finding a smear layer removal agent which will have the efficacy of EDTA combatting its disadvantages is still on.

There is limited published scientific data regarding smear layer removing agents because of their own advantages and disadvantages. Apple cider vinegar is as a common household product has smear layer removing potency due to its components such as acetic, citric, formic, lactic, succinic (succinate), and tartaric acids. Saline which usually has no role to play in removal of smear layer, still has been used as a negative control group because of its usage as a final flush in practice. Therefore, this study was planned to evaluate the efficacy of Apple Cider Vinegar in smear layer removal with 17% EDTA and Saline, as final irrigating solutions using scanning electron microscopy.

Materials and Methods

Selection of samples: Fifteen freshly extracted straight single rooted human maxillary central incisors were collected. Inclusion criteria were permanent teeth, with intact apices, no previous endodontic treatment and small restoration. Exclusion criteria were root length shorter than 17 mm, extensive restoration, root caries, cracks and fracture.

Specimens were decoronated at the level of CEJ by diamond disc to get root of 17 mm. Root canals were instrumented using step back technique with K-files. During instrumentation, copious irrigation was done with 1 ml of 2.5% Sodium Hypochlorite. Upon completion of canal preparation, apexes were sealed with wax to prevent extrusion during final irrigation.

The samples are divided into Groups I, II, and III containing 5 samples each.

Group I- 17% EDTA irrigation.

Group II- Apple Cider Vinegar

Group III- 0.9% Normal Saline.

Then each sample was irrigated with 5 ml of each irrigant for 1 min. All the irrigants were freshly prepared and standardized. According to the groups, irrigants were delivered into the root canals with a side vented Dr.Omkar Eswara Babu Danda, et al. International Journal of Dental Science and Innovative Research (IJDSIR)

endodontic irrigating needle (RC Twents, Prime Dental Products, Mumbai, India) until the working length using manual technique.

Sample preparation for SEM analysis

The samples were sectioned longitudinally with diamond disk and split using a chisel and mallet. One half of each sample is selected and prepared for SEM examination. The specimens were dehydrated using ethyl alcohol: 30% for 10 min, 50% for 20 min, 70% for 20 min, 90% for 30 min and 100% for 30 min. After that the specimens were mounted on coded stubs, air dried, placed in a vacuum chamber, and sputter-coated with a 300 A gold layer. The specimens were then analyzed using a SEM (Cam scan MV 2300, Oxford Instrument, UK). The dentinal surfaces were observed at cervical, middle, and apical thirds with a magnification of $\times 2,000$ for the presence/absence of smear layer and visualization of the entrance to dentinal tubules. Photomicrographs ($\times 2,000$) of these areas on each of the coronal, middle and apical thirds were taken [Figures1-3].



Figure 1: (a) SEM image of group I at coronal third. (b) SEM image of Group I at middle third. (c) SEM image of Group I at apical third. SEM = Scanning electron microscope



Figure 2: (a) SEM image of Group II at coronal third. (b) SEM image of Group II at midddle third. (c) SEM image of Grup II at apical third



Figure 3

(a) SEM image of Group III at coronal third.(b) SEM image of Group III at middle third.(c) SEM image of Group III at apical third

The scores were attributed according to the rating system developed by Torabinejad *et al.*:[6]

No smear layer (no smear layer on the surface of the root canal: All tubules were clean and open).

Moderate smear layer (no smear layer on the surface of the root canal, but tubules contained debris).

Heavy smear layer (smear layer covered the root canal surface and the tubules).

Statistical Methods

On the whole, assessment was done involving coronal, middle and apical portion. The data was analyzed using SPSS (Statistical Package for Social Sciences) version 15.0. Ordinal data was analyzed by nonparametric Kruskal-Wallis statistical test at significance level of $P \leq$ 0.05.

Results

All irrigants tested, have removed smear layer effectively from coronal and middle third. At the apical third, 0.9% normal saline showed poor smear layer removing property, but EDTA (Group I) and apple cider vinegar (Group II) showed comparatively better results at the apical third [Table 1]. There was significant difference between the groups (P < 0.05) [Table 2]. Intragroup comparison can be considered to show significant difference in coronal against apical region in EDTA group, significant difference in coronal vs apical and middle vs apical in apple cider vinegar group and a

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significant change in coronal-middle and coronal-apicalregions in 0.9% normal saline [Table 3].Table 1: Mean smear layer removal by different irrigants at coronal, middle, and apical levels.

Groups	Coronal	Middle	Apical
17% EDTA	1	2	1
Apple Cider Vinegar	1	2	1
Saline	3	3	3

Table 2: Intergroup and Intragroup comparisons

SOURCE OF VARIATION	SS	df	MS	F	P-value	F crit
Between Groups	40.933	2	20.4667	11.80769	0.001463	3.74894
Within Groups	20.8	12	1.7333			
Total	61.7333	14				

Table 3: Intergroup comparisons of EDTA, Apple Cider Vinegar and Normal Saline

Groups	Smear Layer Scoring		Sum	Average	Variance
1	Mean	5.2	26	1.733	0.462
(17% EDTA)	Std. deviation	1.6432			
2	Mean	5.4	27	1.8	0.427
(Apple Cider Vinegar)	Std. deviation	1.5166			
3	Mean	8.8	44	2.933	0.062
(Saline)	Std. deviation	0.4472			

Discussion

The success of endodontic treatment depends on removal of certain vital and necrotic debris, microorganisms and their by-products. Smear layer which contains both organic and inorganic components, has been recommended for removal from the canal walls. Since it can act as a barrier between filling materials and the canal wall it results in compromising the formation of a satisfactory seal. It is generally accepted that the complete removal of the smear layer from root canal walls induces penetration of the root canal sealers into the root dentin, thereby decreasing micro-leakage. The use of a final irrigating solution in endodontic treatment diminishes the inefficiency of sodium hypochlorite irrigation during instrumentation. This course of action could enhance its capacity to act on the mineral matrix of the tooth and act in removing the smear layer formed during biomechanical preparation. Studies have shown that many irrigating solutions have been tested for removal of smear layer

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efficiently. Over the years the use of ethylene diamine tetra-acetic acid (EDTA) was well appreciated in removing the smear layer. It has been found that a final irrigation of EDTA can open up the dentinal tubules, and thus it increases the number of lateral canals to be filled. However, EDTA can cause erosion of dentin, depending on its application time and concentration. 1%, 5%, 10%, and 15% of EDTA when used for 1 min caused erosion of the root dentin [5]. In due course of time, it was found to exhibit various drawbacks. This paved way to search for a final irrigating solution that was more efficient and biocompatible than the EDTA.

Apple cider vinegar, which is a common household product has smear layer removing potency due to its components such as acetic, citric, formic, lactic, maleic, succinic (succinate), and tartaric acids. Saline which usually has no role to play in removal of smear layer, still has been used as a negative control group because of its usage as a final flush in practice. Therefore, our study was planned to evaluate and compare efficacy of Apple Cider Vinegar in smear layer removal with 17% EDTA and Saline, as final irrigating solutions using scanning electron microscopy. In the present study, straight single-rooted and single canal maxillary central incisors were selected, in order to avoid anatomic variation and to maintain standardization. Standardized step-back technique was followed for preparation of root canal using K-files up to size 50 reaching full working length, which eventually facilitates penetration of irrigating solutions to the apical third producing a greater reduction in remaining bacteria and dentin debris as compared with smaller preparations. Then the samples were prepared for score analysis under scanning electron microscope. Other than SEM, the smear layer can also be scored by using digital image analysis. It can overcome the potential evaluator bias, requires less time, and other parameters of interest like density and average diameter of dentinal tubules can be measured; but SEM was still opted in this study because it is a commonly available, tried and tested tool for evaluating the smear layer [7]. When tested, they have removed smear layer effectively from coronal and middle third. But EDTA (Group I) and apple cider vinegar (Group II) showed comparatively better results at the apical third. In addition, the difference found to be significant between groups. Normal saline which was used as a negative control group had inefficient role to play in removal of smear layer. 17% EDTA and Apple Cider Vinegar have exhibited similar scores and efficiency in removing smear layer. Moreover, the dentin in the apical third of the root canal is sclerosed; hence, EDTA may not have such a pronounced action on sclerosed dentin in apical third [8]. Apple cider vinegar has been reported outstanding because of its antimicrobial action, and smear layer removal even in the apical third of the root canal.

Another possible reason is maleic acid which is a component of apple cider vinegar has been studied previously with regard to its efficacy on removal of smear layer. There it has been shown to be more effective than EDTA in removing the smear layer , which may contribute to better adhesion of root canal sealers to root dentin, thus decreasing microleakage [7][9]. It was reported that maleic acid when used at 7% was effective in removing the smear layer[10]. Thus this paved the way for studying the apple cider vinegar, which is quite active against endodontic microorganisms to see the ability to remove the smear layer, and also to compare directly with EDTA, which is still mostly used for smear layer removal.

Conclusion

Apple cider vinegar has comparable smear layer removal efficacy with 17% EDTA solution from the coronal, middle and apical third of the root canals. Within the limitations of this study, it may be concluded that apple

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cider vinegar as a final irrigant has the potential to remove smear layer from the root canal in comparison to normal saline.

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